

10/08/2009

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

These amendments introduce no new matter and support for the changes is replete throughout the specification, claims, and drawings as originally filed. All changes are made without prejudice and are not to be construed as abandonment of any previously claimed subject matter or agreement with any objection or rejection of record.

Listing of Claims:

1-37. (Cancelled)

38. (Currently amended) A composition comprising a recombinant mutant GAL4 protein, or portion thereof, in a eukaryotic cell, wherein the mutant GAL4 protein, or portion thereof, comprises at least one unnatural amino acid selected from the group consisting of a p-acetyl-L-phenylalanine (1), p-benzoyl-L-phenylalanine (2), p-azido-L-phenylalanine (3), O-methyl-L-tyrosine (4), and p-iodo-L-phenylalanine (5) and wherein the GAL4 protein, or portion thereof is at least 90% identical to a GAL4 protein encoded by a polynucleotide amplified from vector pCL1 using a forward PCR primer comprising a nucleotide sequence set forth in SEQ ID NO: 103 and a reverse PCR primer comprising a nucleotide sequence set forth in SEQ ID NO: 104 that comprises a full-length wild-type N-terminal DNA binding domain and a full-length wild-type C-terminal activation domain, and wherein the recombinant mutant GAL4 protein is capable of activating a GAL4 responsive gene.

39. (Previously Presented) A composition comprising a recombinant protein, wherein the protein comprises at least one p-acetyl-L-phenylalanine, p-amino-L-phenylalanine, or p-azido-L-phenylalanine comprising at least one post-translational modification, wherein the at least one post-translational modification comprises a saccharide moiety, and wherein the protein additionally comprises an oligosaccharide covalently coupled to an asparagine, threonine or serine residue of the protein.

40. (Cancelled)

41. (Original) The composition of claim 39, wherein the at least one post-translational modification is made in vivo in a eukaryotic cell.

42. (Currently amended) A composition comprising a protein and an excipient, wherein the protein comprises at least one p-acetyl-L-phenylalanine, p-amino-L-phenylalanine, or p-azido-L-phenylalanine and at least one post-translational modification that is made in vivo by a eukaryotic cell, wherein the post-translational modification is not naturally made by a prokaryotic cell, wherein the modification is selected from the group consisting of: phosphorylation, lipid-modification, palmitoylation, palmitate addition and a glycolipid-linkage modification ~~and an excipient~~.

43-46. (Cancelled)

47. (Previously Presented) The composition of claim 42, wherein the protein further comprises an additional post-translational modification selected from the group consisting of: glycosylation, acetylation, acylation, lipid-modification, palmitoylation, palmitate addition, phosphorylation, and glycolipid-linkage modification.

48-49. (Cancelled)

50. (Original) The composition of claim 42, wherein the protein comprises at least two unnatural amino acids.

51. (Original) The composition of claim 50, wherein the protein comprises at least two different unnatural amino acids.

52. (Original) The composition of claim 42, wherein the protein comprises at least three unnatural amino acids.

53. (Original) The composition of claim 42, wherein the protein comprises four or more unnatural amino acids.

54. (Original) The composition of claim 42, wherein the composition further comprises a pharmaceutically acceptable excipient.

55. (Original) The composition of claim 42, wherein the composition comprises at least 100 micrograms of the protein.

56. (Original) The composition of claim 42, wherein the composition comprises at least 50 µg/liter of the protein.

57. (Original) The composition of claim 42, wherein the protein comprises a secretion or localization sequence, an epitope tag, a FLAG tag, a polyhistidine tag, or a GST fusion.

58-130. (Cancelled)

131. (Previously Presented) A protein produced by a method comprising the steps of: growing, in an appropriate medium, a eukaryotic cell that comprises a nucleic acid that comprises at least one selector codon and encodes the protein; wherein the medium comprises p-acetyl-L-phenylalanine, p-amino-L-phenylalanine, or p-azido-L-phenylalanine,

and the eukaryotic cell comprises:

an orthogonal tRNA (O-tRNA) that functions in the cell and recognizes the selector codon; and

an orthogonal aminoacyl tRNA synthetase (O-RS) that preferentially aminoacylates the O-tRNA with p-acetyl-L-phenylalanine, p-amino-L-phenylalanine, or p-azido-L-phenylalanine,

wherein the protein is modified by at least one post-translational modification in vivo and wherein the post-translational modification is selected from the group consisting of: lipid-modification, palmitoylation, palmitate addition and a glycolipid-linkage modification.

132. (Previously Presented) The protein of claim 131, wherein the protein is further modified through the p-acetyl-L-phenylalanine, p-amino-L-phenylalanine, or p-azido-L-phenylalanine.

133. (Previously Presented) The protein of claim 131, wherein the protein is modified by at least one post-translational modification to the p-acetyl-L-phenylalanine, p-amino-L-phenylalanine, or p-azido-L-phenylalanine in vivo and wherein the post-translational modification is selected from the group consisting of: N-glycosylation, O-glycosylation,

acetylation, acylation, lipid-modification, palmitoylation, palmitate addition, phosphorylation, and glycolipid-linkage modification.

134-143. (Cancelled)

144. (Previously Presented) The recombinant mutant GAL4 protein of claim 38, wherein the recombinant mutant protein is at least 95% identical to a GAL4 protein encoded by a polynucleotide amplified from vector pCL1 using a forward PCR primer comprising a nucleotide sequence set forth in SEQ ID NO: 103 and a reverse PCR primer comprising a nucleotide sequence set forth in SEQ ID NO: 104.

145. (Previously Presented) The recombinant mutant GAL4 protein of claim 38, wherein the recombinant mutant protein is at least 99% identical a GAL4 protein encoded by a polynucleotide amplified from vector pCL1 using a forward PCR primer comprising a nucleotide sequence set forth in SEQ ID NO: 103 and a reverse PCR primer comprising a nucleotide sequence set forth in SEQ ID NO: 104.

146. (Previously Presented) The recombinant mutant GAL4 protein of claim 38, wherein the recombinant mutant GAL4 protein comprises a wild-type DNA binding domain encoded by a polynucleotide amplified from vector pCL1 using a forward PCR primer comprising a nucleotide sequence set forth in SEQ ID NO: 103 and a reverse PCR primer comprising a nucleotide sequence set forth in SEQ ID NO: 104.

147. (Previously Presented) The recombinant mutant GAL4 protein of claim 38, wherein the recombinant mutant GAL4 protein comprises a wild-type activation domain encoded by a polynucleotide amplified from vector pCL1 using a forward PCR primer comprising a nucleotide sequence set forth in SEQ ID NO: 103 and a reverse PCR primer comprising a nucleotide sequence set forth in SEQ ID NO: 104.

148. (Cancelled)

149. (Previously presented) The recombinant protein of claim 39, wherein the protein comprises between 1 and 10 unnatural amino acid residues.

150. (Previously presented) The recombinant protein of claim 39, wherein the protein comprises between 1 and 5 unnatural amino acid residues.

151. (Previously presented) The composition of claim 42, wherein the protein comprises an amino acid sequence that is derived from a naturally occurring erythropoietin (EPO), IFN-beta, Factor VII, Factor VIII, or antibody.